Injury to *Staphylococcus aureus* During Sausage Fermentation

J. L. SMITH AND S. A. PALUMBO*

Eastern Regional Research Center, Federal Research, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118

Staphylococcus aureus 196E added to a beef sausage containing starter culture and 0.5 to 2.0% glucose and incubated at 35°C was unable to grow when plated on tryptic soy agar (TSA) containing 7.5% NaCl. The injury, presumed to be due to the lactic acid produced during fermentation, was more pronounced at the lower concentrations of glucose (and lower acid levels). In the absence of glucose and/or starter culture, no injury was observed. When sausages containing *S. aureus* injured by fermentation at 35°C were incubated at 5°C, the counts on TSA (measures both injured and uninjured cells) and TSA containing 7.5% NaCl (measures uninjured cells only) remained constant; however, upon reincubation of the cold-stored sausage at 35°C, the staphylococcus counts on TSA and TSA containing 7.5% NaCl and were similar to the counts of *S. aureus* present in fermented sausages that had never been subjected to 5°C. The demonstration of acid injury indicated that the injury phenomenon must be considered when determining numbers of viable *S. aureus* in fermented sausages.

A great deal of attention has been focused on injury to microorganisms subjected to sublethal adverse conditions, particularly those of practical significance to the food industry (1). Unfortunately for food technologists, most of the research on injury to the commonly found *Staphylococcus aureus* has been carried out with suspensions in phosphate buffer or bacterial culture media rather than in a food environment. The few publications on injury to *S. aureus* in foods that have appeared reported heat injury during the heating of milk (2, 4, 10, 11), during cheese manufacture (13), and during frankfurter processing (9).

No reports have been found concerning injury to *S. aureus* during sausage fermentation. Therefore, we studied the extent of injury to *S. aureus* 196E during the fermentation step in the manufacture of sausages similar to lebanon bologna, summer sausage, or pepperoni.

MATERIALS AND METHODS

Preparation of fermented sausages. Sausages were prepared by the procedures described by Palumbo et al. (8). The formulation, based on 1 kg of beef, was: 30 g of NaCl, 0 to 20 g of glucose, 8.1 g of purified spice mixture (Griffith Laboratories, Union, N.J.), 0.156 g of $NaNO_2$, and 0 or 4 ml of diluted Lactacel MC starter culture (Merck & Co., Inc., Rahway, N.J.). Beef was ground through a 12.7-mm plate, and the above ingredients were mixed in. The mixture was then ground through a 3.18-mm plate and stuffed into 55-mm fibrous moisture-proof casings (Union Carbide Corp., Chicago, Ill.). The fermentation temperature was 35°C.

Preparation of sausages containing staphylococci. A 1-ml amount from a 24-h tryptic soy broth (Difco Laboratories, Detroit, Mich.) culture of *S. aureus* 196E was inoculated into 100 ml of tryptic soy broth contained in a 1,000-ml Erlenmeyer flask. The flask was incubated on a rotary shaker (200 rpm) for 24 h at 35°C. A total of 40 ml from the shaken flask culture was added to 3 kg of sausage mixture, and the bacterial cells were mixed in by hand kneading (approximately 10^7 staphylococci per g of sausage) before the meat mixture was stuffed into casings.

Enumeration of *S. aureus*. At various time intervals, 50 g was removed aseptically from a sausage and placed in a sterile polyethylene bag, 200 ml of sterile 0.1% peptone (Difco) water was added, and the mixture was blended for 3 min in a Stomacher 400 blender (Cooke Laboratory Products, Alexandria, Va.). The blended sample was used to determine the surviving *S. aureus* by direct surface plating of the appropriate dilutions on tryptic soy agar (TSA; Difco) and TSA plus 7.0% NaCl (TSAS) (12) and by the MPN method (brain heart infusion broth enrichment followed by plating on Baird-Parker agar) described by Palumbo et al. (9). The lower limit of detection for the plating medium was 25 cells per g, and that of the MPN method was 0.3 cell per g.

Determination of pH and percent lactic acid. A 50-g amount of control sausage (lacking *S. aureus*) was placed in a polyethylene bag, 200 ml of distilled water was added, and the mixture was blended 3 min in the Stomacher blender. Percent lactic acid and pH were determined for the meat slurry by the method of Zaika et al. (14).

RESULTS

Injury to *S. aureus* as a result of acid production in fermented sausages was measured by the TSA-TSAS plating method of Stiles and Witter (12). Both injured and noninjured cells grow on TSA plates but only noninjured cells grow on TSAS. When survival curves were plotted, the difference between the respective counts of cells on TSA and TSAS at any time increment represented the number of injured cells.

Injury to *S. aureus* 196E occurred during sausage fermentation when glucose (0.5 to 2.0%) and starter culture were present (Fig. 1, 2, and 3); the count on TSAS dropped more rapidly than did that on TSA. The number of injured cells was greater at the lower concentrations of glucose.

Both glucose and starter culture were necessary to produce injury to *S. aureus* during sausage manufacture. In the absence of glucose (but in the presence of starter culture) little or no injury was apparent (Fig. 4). Similar results were

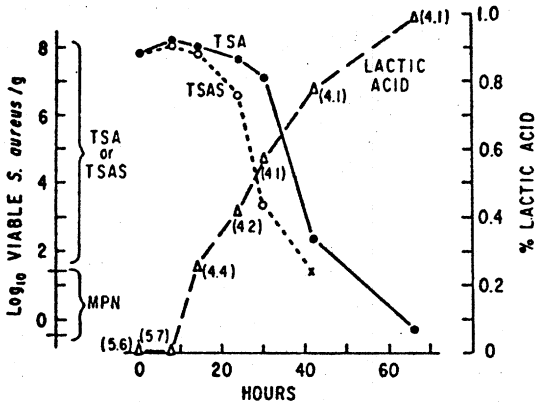

FIG. 1. *Acid injury to S. aureus 196E during fermentation of sausage with 2% added glucose. Numbers in parentheses are pH values; × is count at or below lower limit of detection.*

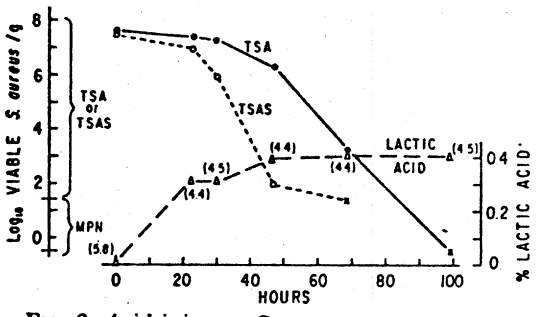

FIG. 2. *Acid injury to S. aureus 196E during fermentation of sausage with 1% added glucose. Numbers in parentheses are pH values; × is count at or below lower limit of detection.*

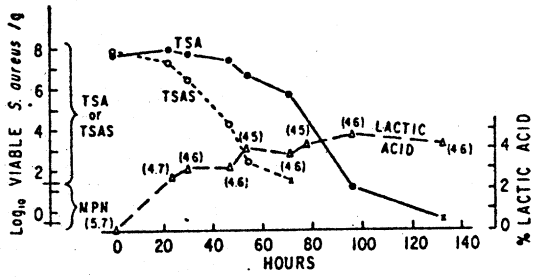

FIG. 3. *Acid injury to S. aureus 196E during fermentation of sausage with 0.5% added glucose. Numbers in parentheses are pH values; × is count at or below the lower limit of detection.*

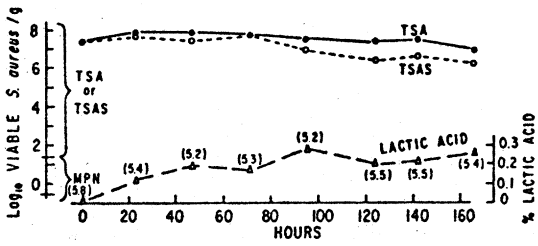

FIG. 4. *Fate of S. aureus 196E during sausage fermentation with no added glucose. Numbers in parentheses are pH values.*

observed in the presence of glucose (but absence of starter culture) or in the absence of both starter culture and glucose.

In one series of experiments, sausages containing *S. aureus* and 1% glucose (with starter culture) were allowed to ferment at 35°C for about 48 h so that substantial injury occurred (Fig. 5A). Then six sausages were placed at 5°C for several days. The counts of *S. aureus* 196E on TSA or TSAS remained essentially unchanged during cold storage (Fig. 5B), but decreased when the temperature of incubation of the sausages was changed from 5 to 35°C (Fig. 5C).

Large numbers of *S. aureus* cells were used to facilitate detection and enumeration of injured cells. In raw beef, the level of *S. aureus* was found to be 3.2×10^3 cells per g (7). When lower and more realistic numbers of staphylococci were used (10^3 cells per g), injury was demonstrated (Table 1), but the injury was much less than that observed when 10^7 cells per g were initially present in sausages containing 1% added glucose (Fig. 2). At the lower *S. aureus* count, the numbers decreased to an undetectable level (<0.3 cell per g) at approximately 40 h, but with 10^7 cells per g approximately 100 h of incubation were necessary before the viable cells decreased to the undetectable level.

Growth of *S. aureus* 196E was not observed in any of the experimental sausages. Staphylo-

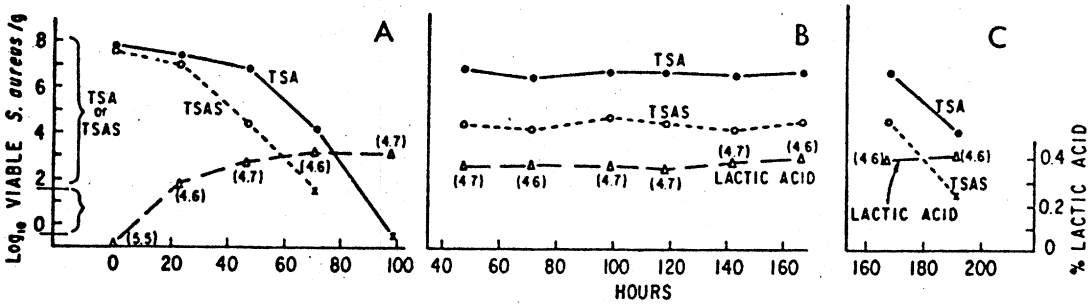

FIG. 5. *Influence of low-temperature storage on S. aureus 196E injured during sausage fermentation with 1% added glucose. Numbers in parentheses are pH values; × is count at or below lower limit of detection. (A) All sausages incubated at 35°C. (B) At 47 h, seven sausages were removed from 35°C and placed at 5°C. (C) At 167 h, one sausage was removed from 5°C and placed at 35°C.*

TABLE 1. *Fate of low numbers of S. aureus 196E during sausage fermentation with 1% added glucose*

Time (h)	Viable counts per g on:		% Lactic acid	pH
	TSA	TSAS		
0	1.3×10^3	9.8×10^2	0	5.8
22.5	3.7×10^2	2.5×10^1	0.49	4.4
46.5	<0.3[a]	$<2.5 \times 10^1$	0.54	4.5

[a] Determined by MPN method.

cocci present at approximately 10^7 cells per g were unable to increase in numbers in the absence or presence of glucose and/or starter culture. In all sausages containing glucose and starter culture, the numbers of staphylococci decreased to undetectable levels (<0.3 cell per g). At 2% glucose and an initial *S. aureus* level of 10^7 cells per g, no viable cells were detected at approximately 60 h (Fig. 1), and with 0.5% glucose, no viable cells were detected at approximately 130 h (Fig. 3); thus, the rate of killing in the acid environment increased concomitantly with the increase in the glucose concentration.

DISCUSSION

Previous work (9) has shown that the heating step of the frankfurter process can injure *S. aureus* added to the emulsion. In the present study, we show that the acid produced by lactic acid starter culture bacteria during the fermentation step in dry and semidry sausage processing can lead to injury of *S. aureus* present in the sausages.

Acid injury to *S. aureus* was related to the initial level of glucose added to the sausage because the glucose level governed the amount of acid produced by the starter cultures. Acid injury was greater in sausages prepared with 0.5% glucose than in those prepared with 2% glucose. At the higher glucose levels, the starter culture produced large amounts of lactic acid quickly so that the injury effect was minimal and transient; the killing effect of the acid overrode the injury effect. However, at lower concentrations of glucose, the staphylococci were in contact with nonlethal but injurious levels of acid; thus, the injury effect was prominent and longlasting before the damage led to death of the cells. Injury and death were not observed in the absence of glucose or starter culture.

Sausages such as lebanon bologna contain high levels of fermentable carbohydrates and undergo a long fermentation time of 2 to 4 days (8). The large amounts of acid produced in lebanon bologna should kill any *S. aureus* present, and very few injured cells should be present. However, in other sausages, such as summer sausage or thuringer, lower levels of fermentable sugars are present, and the fermentation time may be less than 24 h (6); thus, the acid production will be lower, and the sausages may contain viable, but injured, *S. aureus* that are not readily detected when a salt-containing medium is used. Such low-acid sausages pose a threat from *S. aureus*; low levels of acid will not readily kill staphylococci and may lead to considerable acid injury.

Acid-injured cells present in fermented sausages showed no further decrease in TSA or TSAS counts upon shifting the sausages from 35°C to 5°C. Thus, no further death or injury occurred at the lower temperature; but when sausages were shifted back to 35°C, both the TSA and TSAS counts decreased to values similar to that of control sausages that had never been held at 5°C. If it can be assumed that metabolic activity is minimal at 5°C, then injury and death due to acid can occur only when the cells are metabolizing.

The real danger with injured *S. aureus* present in fermented sausages is the transfer of the injured cells from the stressed food environment to other foods via knives, slicers, or by personnel handling such sausages. Transferred to a food which is neutral in reaction (or only slightly

acid) and rich in nutrients, the injured staphylococci may recover, grow, and produce toxin. And indeed, *S. aureus* has been shown to recover the ability to grow and produce enterotoxin after injury (3, 5). However, it is probable that injured *S. aureus* will not be repaired as long as they are in the acid environment of the sausage, even if the sausage is subjected to temperature abuse (temperatures at which staphylococci can grow). When cold-stored sausages containing injured cells were removed to a temperature suitable for the growth of staphylococci, injury to the cells continued and eventually led to death (Fig. 5).

The data presented here indicate that salt-containing isolation or detection media for *S. aureus* cannot give a true picture of the numbers of potentially harmful staphylococci present in stressed foods. Because the phenomenon of injury does occur with *S. aureus* present in fermented foods, media must be used that will permit the detection of both injured and noninjured cells.

ACKNOWLEDGMENTS

We thank Griffith Laboratories, Inc. for their gift of purified spices and Merck & Co., Inc. for starter culture.

LITERATURE CITED

1. **Busta, F. F.** 1976. Practical implication of injured microorganisms in food. J. Milk Food Technol. 39:138–145.
2. **Busta, F. F., and J. J. Jezeski.** 1963. Effect of sodium chloride concentrations in an agar medium on growth of heat-shocked *Staphylococcus aureus*. Appl. Microbiol. 11:404–407.
3. **Collins-Thompson, D. L., A. Hurst, and H. Kruse.** 1973. Synthesis of enterotoxin B by *Staphylococcus aureus* strain S6 after recovery from heat injury. Can. J. Microbiol. 19:1463–1468.
4. **Firstenberg-Eden, R., B. Rosen, and C. H. Mannheim.** 1977. Death and injury of *Staphylococcus aureus* during thermal treatment of milk. Can. J. Microbiol. 23:1034–1037.
5. **Fung, D. Y. C., and L. L. VandenBosch.** 1975. Repair, growth, and enterotoxigenesis of *Staphylococcus aureus* S-6 injured by freeze-drying. J. Milk Food Technol. 38: 212–218.
6. **Goepfert, J. M., and K. C. Chung.** 1970. Behavior of *Salmonella* during the manufacture and storage of a fermented sausage product. J. Milk Food Technol. 33: 185–191.
7. **Goepfert, J. M., and H. U. Kim.** 1975. Behavior of selected food-borne pathogens in raw ground beef. J. Milk Food Technol. 38:449–452.
8. **Palumbo, S. A., J. L. Smith, and S. A. Ackerman.** 1973. Lebanon bologna. I. Manufacture and processing. J. Milk Food Technol. 36:497–503.
9. **Palumbo, S. A., J. L. Smith, and J. C. Kissinger.** 1977. Destruction of *Staphylococcus aureus* during frankfurter processing. Appl. Environ. Microbiol. 34:740–744.
10. **Reiter, B., B. G. Fewins, T. F. Fryer, and M. E. Sharpe.** 1964. Factors affecting the multiplication and survival of coagulase positive staphylococci in cheddar cheese. J. Dairy Res. 31:261–272.
11. **Smolka, L. R., F. E. Nelson, and L. M. Kelley.** 1974. Interaction of pH and NaCl on enumeration of heat-stressed *Staphylococcus aureus*. Appl. Microbiol. 27: 443–447.
12. **Stiles, M. E., and L. D. Witter.** 1965. Thermal inactivation, heat injury, and recovery of *Staphylococcus aureus*. J. Dairy Sci. 48:677–681.
13. **Tuckey, S. L., M. E. Stiles, Z. J. Ordal, and L. D. Witter.** 1964. Relation of cheese-making operations to survival and growth of *Staphylococcus aureus* in different varieties of cheese. J. Dairy Sci. 47:604–611.
14. **Zaika, L. L., T. E. Zell, J. L. Smith, S. A. Palumbo, and J. C. Kissinger.** 1976. The role of nitrite and nitrate in Lebanon bologna, a fermented sausage. J. Food Sci. 41:1457–1460.